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Survival after total body irradiation: Effects of irradiation of exteriorized small intestine

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Summary

Rats receiving lethal irradiation to their exteriorized small intestine with pulsed 18 MVp bremsstrahlung radiation live about 4 days longer than rats receiving a dose of total-body irradiation (TBI) causing intestinal death. The LD₅₀ for intestinal irradiation is approximately 6 Gy higher than the LD₅₀ for intestinal death after TBI. Survival time after exteriorized intestinal irradiation can be decreased, by adding abdominal irradiation. Adding thoracic or pelvic irradiation does not alter survival time. Shielding of large intestine improves survival after irradiation of the rest of the abdomen while the small intestine is also shielded. The kinetics of histological changes in small intestinal tissues implicate the release of humoral factors after irradiation of the abdomen. Radiation injury develops faster in the first (proximal) 40 cm of the small intestine and is expressed predominantly as shortening in villus height. In the last (distal) 40 cm of the small intestine, the most pronounced radiation effect is a decrease in the number of crypts per millimeter. Irradiation (20 Gy) of the proximal small intestine causes 92% mortality (median survival 10 days). Irradiation (20 Gy) of the distal small intestine causes 27% mortality (median survival > 30 days). In addition to depletion of crypt stem cells in the small intestine, other issues (humoral factors, irradiated subsection of the small intestine and shielding of the large intestine) appear to influence radiation-induced intestinal mortality.

Introduction

In the past, the crypt stem cell of the small intestine has been identified as the critical target for radiation injury of the intestine [2,7,9,26]. In experiments with well-defined moderately homogeneous total-body irradiation (TBI), shielding of short pieces of small intestine improves survival after TBI [1,14,18,22]. The increase in survival time is directly correlated to the length of intestine shielded, and shielding the ileum (distal small intestine) is more effective than shielding the duodenum (proximal small intestine) [22]. The interpretation offered for these results is that the number of crypt stem cells that survive radiation in the small intestine is not the sole determinant of radiation morbidity or mortality. Additional factors appear to modify the intestinal radia-

tion response. The present experiments in rats were designed to test the possibility that (1) humoral substances released by irradiated parts of the body can modify radiation-induced intestinal mortality and (2) different parts of the small intestinal tract have different radiation sensitivities. For these purposes, isolated loops of small intestine with or without other parts of the body received single, high-dose radiation, while the rest of the animal was shielded.

Material and methods

Animals

Specific pathogen free Sprague-Dawley rats 6–8 weeks old and weighing 250–350 g were quarantined on arrival and screened for the absence of disease and *Pseudo-*

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monas spp. before being released. They were maintained in an AAALAC-accredited facility, caged in pairs with filter covers, and provided commercial rat food pellets and acidified tap water (pH 2.5) *ad libitum*. Animal holding rooms were maintained at $21 \pm 2^\circ\text{C}$ with $50 \pm 10\%$ relative humidity using at least 10 air changes per hour of 100% conditioned fresh air. They were on a 12 h light/dark full-spectrum light cycle with no twilight. Lights were on between 7:00 a.m. and 7:00 p.m.

Surgery and anesthesia

General anesthesia was maintained during surgical procedures using ketamine hydrochloride (50 mg/kg) in combination with xylazine hydrochloride (10 mg/kg) given intramuscularly and supplemented as needed. Aseptic procedures were used. A midline abdominal incision was made and the portion of the small intestine to be shielded or irradiated was exteriorized. Hemorrhage was not normally a problem in this surgical procedure; however, if it occurred, 4-0 chromic gut suture material was used to ligate bleeding vessels. The animal was irradiated as described below. After irradiation, the small intestine was repositioned in the abdominal cavity and the abdominal wall was closed using single interrupted 4-0 monofilament nylon sutures. The skin was closed with clamps. Anesthesia was maintained for the entire time (approximately 40 min). Pedal reflexes were monitored to assure maintenance of adequate anesthesia depth. Animals were allowed to regain consciousness in individual cages after completion of the irradiation exposure and surgical closure.

Irradiation

A linear accelerator was operated at 18 MVp, 60 pulses/sec, 4 μsec pulse width, and a nominal dose rate of 10 Gy/min. A bremsstrahlung beam was generated by impinging 18 MeV electrons onto a 4-mm thick water-cooled tantalum target. The average photon energy was 6 MeV. The target to skin distance was 325 cm. The dose per pulse was determined before irradiation of each group of animals. The restraint device allowed for shielding of the exteriorized portion of the small intestine or shielding of different parts of the body with slabs of lead during irradiation. Lead slabs 0.5, 1.0, 2.0, 3.0, or 10.0 cm thick were used. Figure 1 shows the physical setup. The exteriorized intestine was contained in a 30-ml glass beaker filled with Ringer's lactate at a temperature of 37°C . Every radiation exposure was monitored by two ionization chambers in the field that were calibrated to the midline dose in an acrylic rat or intestinal phantom for each irradiation day. The actual dose delivered was $\pm 3\%$ of the prescribed dose.

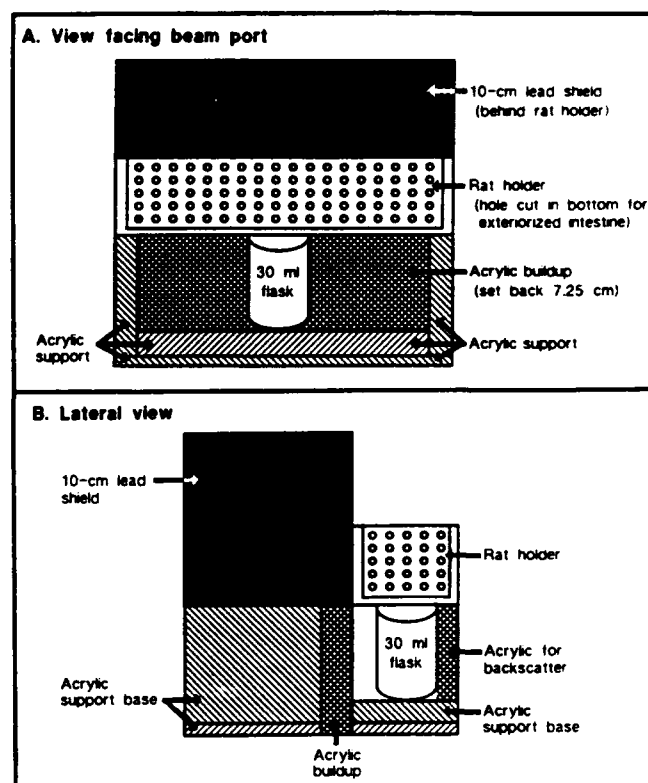


Fig. 1. Physical set up for exposure of small intestine to 18 MeV linear accelerator.

Dose measurements were made with ionization chambers and thermoluminescent dosimeters (TLDs) placed in an acrylic rat or intestinal phantom. Ionization chambers were used to determine the dose rate according to the protocol established by Task Group 21 of the American Association of Physicists in Medicine [19]. The TLDs were used to determine dose uniformity and the effectiveness of the shielding. Single doses between 15 and 60 Gy were used. Doses higher than 60 Gy were not used because of the logistics of working with animal tissue radioactivity induced by neutron activation [27] and the higher doses delivered to the shielded areas. Experiments of a maximum of 12 rats were planned per day and always performed during the same time of day. All animals were irradiated between 10:00 a.m. and 3:00 p.m.

In addition to the exteriorized small intestine, three different 5×5 cm fields, were irradiated: (1) the thorax, which included heart, mediastinum, and lungs; (2) the abdomen, which included liver, gall bladder, spleen, stomach, kidney, adrenal gland, pancreas, and colon; and (3) the pelvis, which includes bladder, rectum, and testes. The irradiated exteriorized small intestine consisted of the entire 80 cm of small intestine that can be exteriorized or the (proximal) first 40 cm,

the middle 40 cm or the last (distal) 40 cm of the small intestine.

Animal care after irradiation

Rats remained in individual cages after irradiation. They were evaluated for appetite, hydration, health status, and diarrhea twice a day during the normal work week and once daily during the weekends. Moribund animals and animals that had completed the experiment (> 30 days after irradiation) were killed by CO₂ inhalation. Approximately 15% of the animals were killed before day 30 when they were considered moribund. The other 85% was found dead on inspection rounds or killed at the completion of the experiments.

Morphometric analysis

Computer-assisted morphometric studies [Bioquant System IV; R&M Biometrics, Inc., Nashville, TN (1985)] were performed on tissues obtained from animals killed at predetermined days. The small intestine samples were taken at 20, 40, and 60 cm of an average 80-cm total length. The samples at 20 cm (proximal) and 60 cm (distal) were selected for complete histological and statistical analysis. The tissues were fixed in 10% buffered formalin. Transverse sections were embedded in paraffin. Five-micron sections were cut, placed on glass slides, and stained with hematoxylin and eosin. At least five slides per animal were analyzed for each sample. Tissues of three or more animals were used for each parameter evaluated: the intestinal circumference, crypts per millimeter, villus height, and the number of cells per crypt-villus length. A crypt containing only necrotic cells (crypt abscess) was not counted. The base of the villus was determined by comparing several adjacent villi and drawing a line through the estimated bases of several villi. Villus height and villus-crypt length were measured in millimeters from the tip to the base of the villus and the muscularis mucosa, respectively. Tissues taken from the proximal and distal small intestines from four experimental groups were analyzed: (1) rats receiving 20 Gy irradiation to 80 cm of exteriorized small intestine, (2) rats receiving 20 Gy of abdominal irradiation while 80 cm of exteriorized small intestine were shielded, (3) rats receiving 20 Gy irradiation to the abdomen and the 80 cm of exteriorized small intestine, and (4) rats receiving anesthesia, surgery, and sham-irradiation.

Statistics

LD₅₀ values were determined by probit analysis (Fig. 2). Differences in means of survival times or morphometric parameters between two experimental groups or between an experimental group and a control

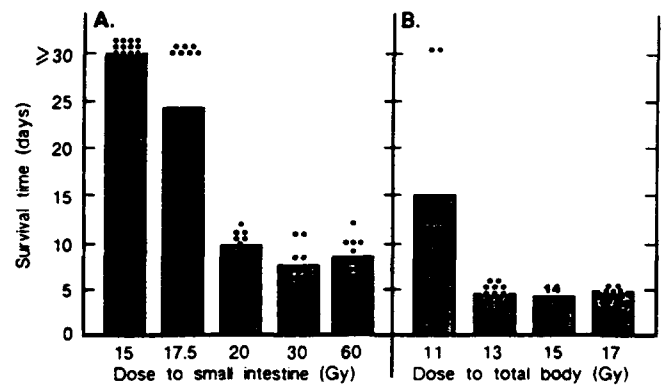


Fig. 2. Influence of small intestinal irradiation or TBI on survival. Survival times of individual animals are shown by closed circles. Bar height indicates mean survival time. Experiments are terminated after day 30.

group were analyzed for statistical significance by the Student's *t*-test (Fig. 2 and Table IV). Nonparametric tests (Mann-Whitney rank test) were used for determining differences between experimental groups with survival times not normally distributed (Figs. 2,3 and 4). For Fig. 5, a linear-regression, analysis was performed. In the analysis of Fig. 6A,B, *t*-tests and an analysis of variance (ANOVA) was performed.

Results

Dosimetry

Tables I and II summarize the TLD studies. The isolated intestinal loop received a moderately homogeneous radiation dose; the ratio between the maximum

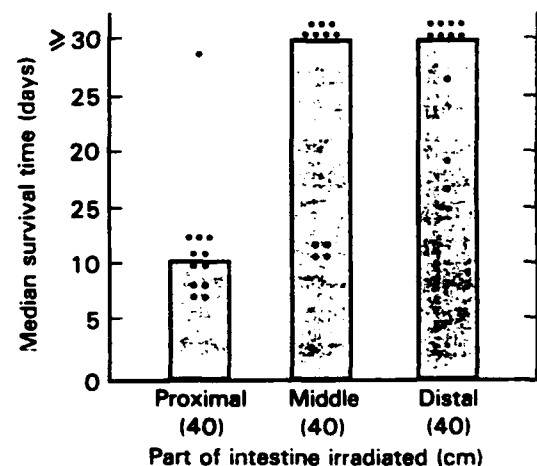


Fig. 3. Influence of partial small intestinal irradiation with 20 Gy on survival. Survival times of individual animals are shown by closed circles. Bar height indicates median survival time. Experiments are terminated after day 30.

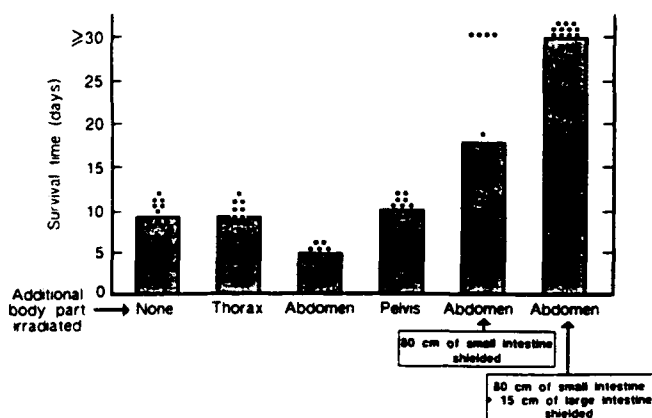


Fig. 4. Survival after a 20 Gy dose of radiation to exteriorized small intestine and irradiation of additional body parts. Survival times of individual animals are shown by closed circles. Bar height indicates mean survival time. Experiments are terminated after day 30.

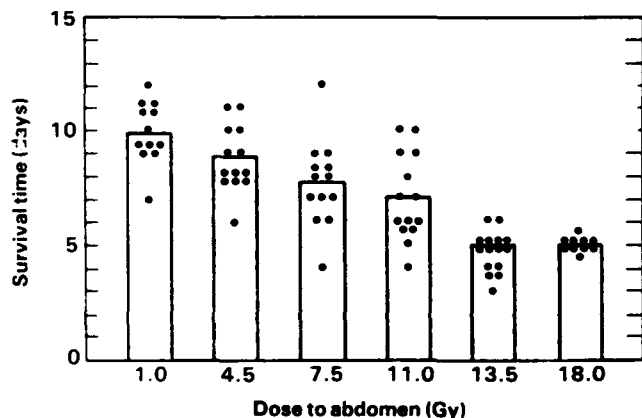


Fig. 5. Influence of the radiation dose received by the abdomen on survival after 20 Gy radiation to exteriorized small intestine. Survival time of individual animals is shown by closed circles. Bar height indicates mean survival time. Experiments are terminated after day 30.

and minimum measured dose was 1.11 (105:95; Table I, last column). The shielding of the rat's body was effective, as 7% or less of the central-axis open-field dose was measured in the shielded rat phantom. Adding an abdominal field of 5×5 cm contiguous to the irradiation field of the exteriorized intestinal loop resulted in an abdominal dose of approximately 90% of the intestinal dose. Shielding appeared to be effective in this array as well. Eight percent or less of the open-field dose was measured behind the shield. Highest doses were found close to the block edges.

Irradiation of exteriorized small intestine

The maximum length of small intestine that can be exteriorized in the rat is approximately 80 cm. The stomach and the first 5–8 cm of the small intestine, the latter due to its retroperitoneal location, cannot be

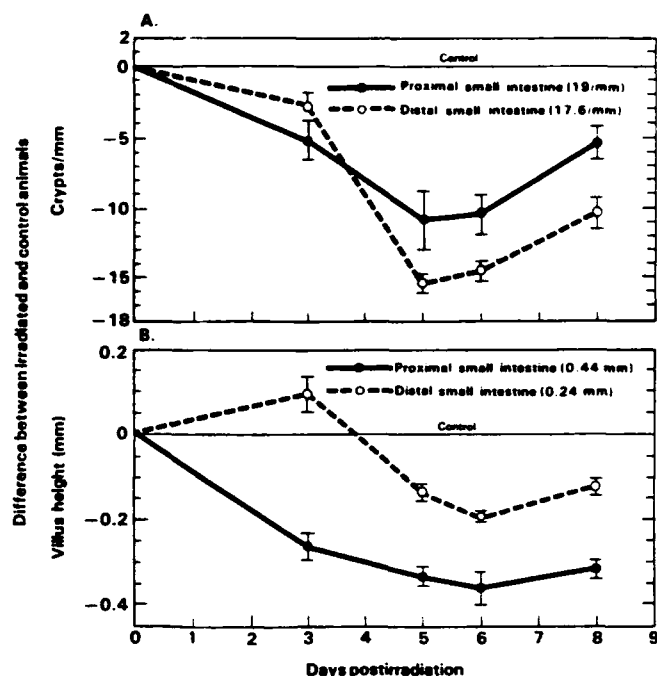


Fig. 6. Changes in the number of crypts/mm (A) and villus height (B) after intestinal irradiation. Mean values with standard error bars are given of the daily mean minus the normal mean (day 0). Day 0 values of control animals are given in parentheses.

exteriorized without irreversibly disturbing the normal anatomy. Figure 2, panel A summarizes the effect of radiation on 80 cm of exteriorized small intestine. For comparison, Fig. 2, panel B also shows results obtained in a previous study of TBI using the same rat model [22]. Irradiating the isolated small intestine caused morbidity (watery diarrhea and lethargy) and mortality at higher radiation doses and at a later time than after TBI.

The $LD_{50/30}$ and 95% confidence limits after exteriorized intestinal irradiation were 17.9 Gy (17.1–19.5). The $LD_{50/6}$ and 95% confidence limits after TBI were 12.3 Gy (12.0–12.9). The $LD_{50/30}$ and 95% confidence limits after TBI (using all the data given in reference 22) were 9.9 Gy (9.0–10.5). Probit analysis for the $LD_{50/6}$ data is of limited value due to 0% or 100% responses for most data. Just averaging the difference between the highest dose leading to a bone marrow syndrome (11 Gy) and the lowest dose leading to an intestinal syndrome (13 Gy) gives a $LD_{50/6}$ of 12 Gy (instead of 12.3 Gy with probit analysis).

The mean survival time after high dose intestinal irradiation was 8.5 days and the mean survival time for the intestinal syndrome after TBI is 5 days. This difference is statistically significant ($p < 0.01$). Thus, the LD_{50} was approximately 5.5 Gy higher for irradiation of the exteriorized small intestine and survival time was approximately 3–4 days longer than after TBI.

TABLE I

Dosimeters in rat phantom and intestinal loop phantom.^a

Thermoluminescent dosimeter placement	Rat body ^b (shielded)		Intestinal loop ^c (irradiated)	
	Location	Dose (%)	Location	Dose (%)
Top → bottom midline field	0.8 ^d	4		
	1.8 ^d	4		
	2.5 ^d	4		
	3.2 ^d	5		
	4.1 ^d	7	0.1 ^d	98
			Central axis (2.5) ^d	100
			4.6 ^d	105
Left → right mid-level phantom	1 ^e (nose)	6		
	3 ^e	5	0.3 ^e	105
	5 ^e	5	0.8 ^e	100
	7.5 ^e (mid)	5	1.5 ^e	100
	9 ^e	5	2.1 ^e	104
	11 ^e	5	2.6 ^e	102
	13 ^e	5		
Entry → exit mid-level phantom	15 ^e (tail)	5		
	0.8 ^f	4	0.3 ^f	100
	1.8 ^f	4	0.9 ^f	101
	2.5 ^f	5	1.5 ^f	100
	3.2 ^f	5	2.1 ^f	97
	4.1 ^f	6	2.6 ^f	95

^a Radiation set up as in Fig. 1. Doses in percent of central intestinal dose.^b Acrylic cylinder (nose end tapered), diameter 5.0 cm, height 16.0 cm.^c Acrylic flat end cylinder, diameter 3.0 cm, height 5.0 cm in glass flask.^d Centimeters from top of midline field.^e Centimeters from left of phantom.^f Centimeters from entry of phantom.

The survival time of animals receiving 20 Gy to their small intestine was longer than the survival of animals receiving 30 Gy ($p < 0.01$). The difference between the 20 and 60 Gy group did not reach statistical significance, presumably due to the limited number of animals tested. In contrast, survival times of rats given TBI of 13, 15, 17, 20, or 30 Gy were not significantly different. Data for the 20-Gy and 30-Gy TBI groups are not shown in Fig. 2. Results in Fig. 3 indicate that the most sensitive part of the small intestine was the proximal 20–40 cm of the small intestine. After 20 Gy the median survival time was 10 days. Irradiation of the middle 40 cm or distal 40 cm with 20 Gy caused mortality significantly later in some animals ($p < 0.001$). However, most of the animals receiving 20 Gy to the middle or distal 40 cm of the small intestine survived longer than 30 days.

Irradiation of small intestine and other body compartments

Different body compartments were irradiated in addition to the whole 80 cm of small intestine. Figure 4 summarizes these results. Thoracic irradiation (5 ×

TABLE II

Dosimetry in rat phantom for irradiating rat abdomen.^a

Thermoluminescent dosimetry placement	Location (cm)	Dose ^b
Abdomen	Top	87
Top → bottom	Bottom	90
Midline (open field)		
Abdomen	1 (nose)	5
Left → right	3	4
at midplane	5	5
Phantom	7	8
(Open field from 8.0–13.0 cm)	9	90
	11	85
	13	27
	15 (tail)	6
Abdomen	Entrance	87
Entry → exit	Exit	85
Midline (open field)		

^a Dosimetry performed in a 5 × 5 cm field with thermoluminescent dosimeters. Radiation set up as in Fig. 1. Phantom same as used in Table I.^b Dose in percent of dose of exteriorized small bowel simultaneously irradiated in extension of 5 × 5 cm abdominal field. Rest of body shielded with 10 cm of lead. Open field values in bold print.

TABLE III

Small intestinal circumference in millimeter \pm SE* after irradiation to abdomen or small intestine.

Experimental group	Proximal small intestine	Distal small intestine
1 Control day 0	9.00 \pm 0.01	9.50 \pm 0.02
2 Abdominal irradiation (20 Gy) small intestine shielded day 5	16.50 \pm 0.10	27.50 \pm 0.10
3 Small intestine irradiation (20 Gy) body shielded day 5	11.50 \pm 0.05	14.00 \pm 0.05

* Three animals per entry, 15 observations; differences between group 1 and 2 are different with a p value of <0.001 in a t -test for proximal as well as distal small intestine.

5 cm field, 20 Gy) or pelvic irradiation (5 \times 5 cm field, 20 Gy) did not change survival time after small intestinal irradiation. However, abdominal irradiation (5 \times 5 cm field, 20 Gy) reduced survival time from approximately 10 days to 5 days and made it similar to survival times after 15 or 20 Gy of TBI. Death before day 10 after irradiation was observed in animals receiving TBI, intestinal irradiation, or partial body irradiation in addition to irradiation of the exteriorized small intestine. Two days preceding the day of death, all irradiated animals showed watery-thin diarrhea and lethargy. Irradiation with 20 Gy of the abdominal field alone with 80 cm of the small intestine exteriorized and shielded caused late mortality (>10 days) in some animals. Their cause of death could not be determined. When, in addition to 80 cm of small intestine, 15 cm of proximal large intestine were exteriorized and shielded and the rest of the abdomen received 20 Gy, all animals survived ≥ 30 days. Irradiation (20 Gy) of the exteriorized large intestine alone did not induce any mortality within

30 days after radiation. Shielding of the large intestine, while the small intestine and the rest of the abdomen received 20 Gy, did not influence the survival time of rats, when compared to animals without large intestinal shielding (data not shown). In separate experiments, the amount of radiation received by the abdomen in the 5 \times 5 cm field was decreased by placing lead slabs 0.5, 1.0, 2.0, 3.0, or 10.0 cm thick in the field. The exteriorized small intestine always received 20 Gy. Figure 5 summarizes the results and shows that the mean survival time correlated inversely with the dose given to the abdomen. By regression analysis a correlation coefficient of -0.94 is found with a p value of <0.01 .

Morphometrics of shielded intestine

The circumference of the shielded small intestine increased after a 20 Gy dose of abdominal radiation. Five days after irradiation, the increase was more pronounced in the distal than in the proximal part of the small intestine (Table III). In the villi of the proximal small intestine, shrinkage was observed (decrease in villus length with increasing numbers of cells per millimeter in comparison to controls), while in the villi of the distal small intestine, elongation and an increased number of cells were observed (concentration of cells per millimeter was same as in control, see Table IV). Smaller differences in the same direction as noted in Tables III and IV were seen on postirradiation days 3, 6 and 8. The day of the largest difference (day 5) was selected for presentation.

Morphometrics of irradiated intestine

Direct irradiation of the exteriorized small intestine caused different histological abnormalities in the proximal and the distal small intestine. In the proximal region, a larger decrease in villus length was observed while, in the distal region, the decrease in the number of crypts per millimeter was more pronounced. This

TABLE IV

Villus height (mm) and cells per villus-crypt after irradiation to abdomen or small intestine.*

Experimental crypt group	Villus height		Cells per villus	
	Proximal intestine	Distal intestine	Proximal intestine	Distal intestine
1 Control day 0	0.44 (0.03)	0.24 (0.01)	58.4 (3.2)	33.6 (1.6)
2 Abdominal irradiation (20 Gy) small intestine shielded day 5	0.15 (0.02)	0.34 (0.04)	25.6 (1.6)	68.8 (4.8)
3 Small intestine irradiation (20 Gy) body shielded day 5	0.13 (0.02)	0.11 (0.02)	16.8 (3.2)	11.2 (2.4)

* Three animals per entry, 15 observations, standard error between brackets. Differences between group 1 and 2 are significant in a t -test at a p value of <0.001 , except for cells per villus crypt in the distal intestine, where p value is <0.01 .

TABLE V

Paradox between effects of irradiation and shielding.*

Experimental protocol	Mean survival time (days)	
	Proximal intestine	Distal intestine
20 Gy radiation to exteriorized small intestine with rest of body shielded	10	> 30
15 Gy TBI and intestinal shielding	6	7

* See text for explanation.

decrease of crypts per mm in the distal region started later than the decrease in villus length in the proximal region; although, for both (crypts per millimeter and villus length) the maximum decrease was found day 5–6 postirradiation. Plotted in Fig. 6A,B are daily means minus the normal mean (day 0) plus and minus one standard error. For villus height (Fig. 6B), normalized distal values were higher than those for normalized proximal values for all time periods ($p < 0.001$). For normalized crypt values (Fig. 6A), there was no significant difference between the distal and proximal values on day 3. P values for the differences on the remaining days (5, 6, 8) were respectively, 0.0186, 0.0069, and 0.001 and indicated significantly less crypts/mm in the distal small intestines on those days.

A 2-way ANOVA was run to determine any significant interaction in Fig. 6A,B. Significance was found in both cases ($p < 0.001$) indicating that distal and proximal values behave differently across time. From Fig. 6A it appears that the difference occurs between days 3 and 5; distal values fall (from normal) faster than proximal values do. From Fig. 6B it appears that the difference occurs between day 0 and 3, in that *proximal* values fall from normal faster than distal values.

To determine what significant changes occur across time for both normalized proximal and distal values, a 1-way ANOVA was run for values on days 3, 5, 6, and 8. In most cases, significant ($p < 0.01$) changes were found. Days 5 and 6 were the exception. No significant changes were found in values for these days.

Discussion

For experiments with TBI as well as with smaller field irradiation, such as isolated loops of the small intestine, well-defined dosimetry is a prerequisite. The results in Tables I and II indicate that the planned doses were received in a moderately homogeneous manner [19].

Therefore, the observed difference in LD_{50} and survival time between TBI and small intestinal irradiation (Fig. 2) are real biological differences and not due to differences in absorbed dose in the intestines under the two different radiation setups. No clinical differences were observed between animals dying after TBI or after small intestines irradiation, indicating that the cause of death was the same in both groups, i.e. fluid and electrolyte loss [6]. Reabsorption of electrolytes and fluid from the irradiated small intestine by the (nonirradiated) colon might play a role in extending survival in animals subjected to small intestinal irradiation. The animals in the last column of Fig. 4 (irradiated abdomen; shielded large intestine) probably provide an example of prolonged survival due to reabsorption of fluids and electrolytes in the large intestine [21]. Previously, other investigators have irradiated exteriorized small intestines of rats using higher doses of radiation and shorter length of intestines (2.5 cm) than used in the present study [12]. Late deaths (between 20 and 100 days after radiation) were observed and proven to be due to radiation-induced intestinal obstruction. This mode of death was not observed in our study due to the indicated differences in experimental design between the studies. Twenty Gy to exteriorized large intestine did not induce mortality within 30 days after irradiation. Shielding of the large intestine did not change the survival time observed after small intestinal plus abdominal irradiation. Reabsorption of fluids in the large intestine appears to be only one of the mechanisms that prolongs survival after small intestinal or abdominal irradiation.

The difference in survival time after small intestinal irradiation or TBI can be attributed to the influence of structure(s) distinct from the small intestine that are only irradiated in the TBI procedure. Blood or lymph vessels feeding or draining the small intestine are irradiated in both cases and cannot explain the difference observed. Larger vessels (aorta, vena cava, and thoracic duct) are only irradiated during TBI. Radiation effects on large vessels are not expected to influence mortality due to intestinal toxicity occurring 5 to 10 days after radiation. By exclusion of other explanations, the release of humoral substances in the irradiated tissues (other than the small intestine) remain as a more probable explanation of the observed results. Such substances would travel to and influence events in the small intestines outside the irradiated field. After abdominal irradiation, the changes in intestinal circumference of the nonirradiated small intestine suggest the release of a humoral factor that decreases muscular tone of the small intestine (see Table III). The results shown in Table IV suggest the presence of humoral factor(s) with different effects on the proliferation of cells in the

nonirradiated proximal and distal small intestine. The influence of dietary factors on intestinal histology after irradiation was excluded by comparison of experimental animals to surgery/anesthesia controls. Eating habits of control and experimental animals receiving intestinal or abdominal irradiation were similar up to day 6 post-irradiation.

Shielding experiments (Fig. 4) exclude bone marrow as the source of the suggested humoral substances that influence mortality from radiation damage to the small intestine. Animals with most of their bone marrow irradiated (pelvis, thorax or both, the latter data not shown) did not die earlier than animals receiving exteriorized small intestinal irradiation only. Tissues other than bone marrow as sources for humoral factors remain to be considered. Radiation itself might change secretion patterns in surviving cells directly. More likely, initial release of humoral factors after radiation is caused by cell death. Due to the short time period after abdominal irradiation in which effects are observed (< 6 days), cells would have to die quickly after irradiation if they are to be the source of humoral factors. Rapid death occurs in high-turnover self-renewal systems or in other specific tissues by the mechanism of apoptosis ("interphase") death. This would implicate lymphocytes, spermatogonia, large intestinal cells, and some types of exocrine cells as potential sources of humoral factors [9,15,16,17]. Testes were included in the pelvic field; irradiating them did not appear to influence survival. Splenic lymphocytes, exocrine glands (like the pancreas and gall bladder) and the stomach were included in the abdominal irradiation field and might be the source of the suggested factors. Studies of irradiated dogs with surgically changed and separated flows of bile, pancreatic secretions, and "intrinsic" intestinal secretions indicated that secretions of the irradiated pancreas can decrease survival time and increase histological changes in the intestinal mucosa after abdominal irradiation [8,11].

An interesting paradox was revealed between proximal and distal small intestine. Early mortality was found only after irradiation of the proximal small intestine (Fig. 3). However, as shown earlier, shielding the distal small intestine prolonged survival more effectively [22]. In Table V, the paradoxical sets of observations are summarized. Radiation of the proximal small intestine causes death earlier. Histologically predominantly a "villus" response is observed (Fig. 6B). After irradiation of the distal small intestine (histologically predominantly a "crypt" response; Fig. 6A), mortality occurs later or not at all. A decrease in villus function (absorption) in the normal small intestines would cause rapid functional deficits and mortality, whereas de-

creases in crypt function (secretion, cell production) in the distal small intestines remain compatible with survival if sufficient villus function remains. The effectiveness of shielding the distal small intestine as evidenced in a preceding paper [7] can be explained by assuming humoral factors from the shielded distal small intestine that influence regeneration in the irradiated proximal small intestine. Such "trophic" factor(s) would have to travel through the bloodstream to affect the proximal small intestine and would be different from the humoral factors released by the irradiated abdomen postulated in this communication. The shielded proximal small intestine could release substances into either the intestinal lumen or the bloodstream that influence the irradiated distal small intestine, but, if so, they would affect the prevention or delay of radiation mortality less significantly. The sites of origin, mode of transportation, and target sites for humoral factors postulated in this communication appear to be similar to suggestions made on totally different data sets summarized in a recent review of intestinal physiology [4]. The reviewed studies explored the adaptation of the small intestine to surgical resection and food diversions and indicated trophic luminal factors coming from the proximal intestine and trophic systemic factors from the distal small intestine.

The crypt stem cell assay as described by Withers and Elkind utilizes the jejunum [26]. Figure 6A indicates that this assay will indicate an "average" radiation damage if taken as representing the radiation response of the whole small intestine. Less damage is seen in proximal crypts (duodenal), more damage in distal crypts (in the ileum). Overall results and in particular the survival times observed in our study (Fig. 3) indicate that the crypt stem cell assay in the jejunum cannot predict morbidity or mortality after intestinal irradiation.

Wheldon and Michalowski postulated that radiation distinguishes between so-called Hierarchical (H) self-renewal systems and Flexible (F) self-renewal systems by the latency period of expression of radiation damage [25]. H-systems would have a fixed latency period independent of radiation dose that is equivalent to the stem cell transit time (the time it takes a stem cell to produce end cells). F-systems would show an inverse relationship between latency period and radiation dose. The small intestines were considered a H-system, with a latency period of 5 days, determined by the time it takes a crypt stem cell to reach the villus tip [2,5]. Results given in Fig. 2 indicate that their proposed discriminatory test for H/F systems identifies the small intestine as an H-system after TBI but as an F-system after small intestinal irradiation. It appears that the proposed use

of radiation to discriminate between H- and F-systems excludes the influence of humoral factors (such as hormones, growth factors, or toxins) on latency periods and cannot be applied to circumstances where those effects can be observed.

Recently, in studies using small rodents delayed deaths were observed after radiation doses that are considered to cause an intestinal syndrome. The investigators changed the previous definition of this endpoint (death occurring up to 6 days after TBI [13]) to death occurring up to 10 days after TBI [10,20]. This definition change was justified by claiming that in specific pathogen free animals the intestinal syndrome can be delayed [10,20]. However, rats in the present study were also specific pathogen-free and died from intestinal damage 5 days after high-dose TBI. Other studies with germ-free mice and totally decontaminated dogs showed that intestinal mortality is delayed only by 1-2 days (not 5 days) and that an increase in LD_{50} of approximately 1.5 Gy is observed [5,9,23]. The extension of the endpoint for intestinal lethality to day 10 after irradiation causes an overlap between intestinal syndrome (death after TBI before day 10) and bone marrow syndrome (death between day 6 and 30 after TBI) lethality; this adjustment can be justified if one could demonstrate experimentally that irradiation of a large volume of bone marrow enhances intestinal toxicity and mortality (not found in this communication; see Fig. 4) or that transplanting bone marrow cells after irradiation prevents or delays death from intestinal radiation toxicity. In fact, this was not observed in several investigations using different species [21,24]. In one of the more recent studies the authors claimed a beneficial influence of bone marrow transplantation on radiation-induced intestinal mortality. However, dosimetric control in the study was insufficient, bone marrow and intestinal mortality could not be separated out appropriately and bone marrow transplantation could only have relieved mortality in animals that were destined to die from bone marrow aplasia [20]. In the other study using a 10-day endpoint of intestinal death sufficient detail to support uniformity of dose distribution was not given [10]. Indeed, the radiation quality and treatment distances reported in both communications produce nonuniform radiation distributions in small rodents [28]. The dosimetry performed in our study indicates that over or under doses to different compartments in the body can change survival patterns

after intestinal radiation. Therefore, we conclude, like others before us [13,21] that with at least a moderately uniform dose distribution and an endpoint selection in laboratory animals based on absorbed dose in the appropriate compartment of the body, no evidence exists for a significant influence of the bone marrow syndrome on the intestinal syndrome.

The implications of the described research are important for clinical management of certain types of radiation accidents, e.g. irradiation to the abdomen. For accurate prognostication, the doses received by the proximal small intestine, the distal small intestine, and the abdomen must be determined separately. This will probably require biological dosimetry given that intestinal organs are distributed diffusely in the abdomen. Presently, the enzyme diamine oxidase (DAO) is a prime candidate for assaying the absorbed dose using intestinal biopsies. However, it would not be suitable for defining doses received by other abdominal organs from which biopsies cannot be taken easily and/or contain very little DAO [3]. In retrospect, the differences observed in response of the proximal and distal small intestine to radiation are not surprising, as their tissues do differ in various other aspects, e.g. morphology, function, and neuroendocrine factors. Further studies are required to explore the mechanisms of radiation damage to different parts of the small intestine, as the radiation-induced intestinal syndrome appears to be more complicated than previously appreciated. New applications of the information obtained will not be limited to radiation protection or radiation oncology, but will also be useful in studies of intestinal physiology and pathology.

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